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Telomere Length, Long-Term Black Carbon Exposure, and Cognitive Function in a Cohort of Older Men: The VA Normative Aging Study

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ABSTRACT

Background: Long-term air pollution exposure has been related with aging-associated cognitive impairment, possibly due to enhanced inflammation. Leukocytes with longer telomere length (TL) are more responsive to inflammatory stimuli, yet TL has not been evaluated in relation to air pollution and cognition.

Objectives: To assess whether TL modifies the association of 1-year exposure to black carbon (BC), a marker of traffic-related air pollution, with cognitive function of older men; and to examine whether this modification is independent of age and C-reactive protein (CRP), marker of inflammation.

Methods: Between 1999-2007, we conducted one to three cognitive examinations of 428 older men in the VA Normative Aging Study. We used covariate-adjusted repeated-measure logistic regression to estimate associations of 1-year BC exposure with relative odds of being a low scorer (≤25) on the Mini-Mental State Examination (MMSE), proxy of poor cognition. Confounders included age, CRP, lifestyle and socio-demographic factors.

Results: Each doubling in BC level was associated with 1.57 (95% CI: 1.20, 2.05) times higher odds of low MMSE scores. The BC-MMSE association was larger only among individuals with longer blood TL (5^{th} quintile) (OR=3.23; 95% CI: 1.37, 7.59; p=0.04 for BC-by-TL-interaction). TL and CRP were neither associated with each other nor with MMSE. However, CRP modified the BC–MMSE relationship, with stronger associations only at higher CRP (5^{th} quintile) and reference TL level (1^{st} quintile) (OR=2.68; 95% CI: 1.06, 6.79; p=0.04 for BC-by-CRP-interaction).

Conclusions: TL and CRP levels may help predict the impact of BC exposure on cognitive function in older men.

INTRODUCTION

Impaired cognition and dementia are a leading cause of loss of independence in daily activities (McGuire et al. 2006), hospitalization (Chodosh et al. 2004), and mortality among older individuals (Bassuk et al. 2000; James et al. 2014). Consistent evidence has linked exposure to air pollution, specifically particulate matter, with poor age-related cognitive performance (Chen and Schwartz 2009; Power et al. 2011; Zeng et al. 2010) and accelerated cognitive decline (Weuve et al. 2012). In particular, exposure to air pollution particles from vehicular traffic, estimated using black carbon (BC) levels, has been associated with poor cognition in older men (Power et al. 2011). The limited availability of biomarkers to identify subsets of the general population that are at higher risk of age-related impaired cognition is a critical public health gap that hampers effective targeted prevention (Sperling et al. 2011). While previous studies have identified a number of factors that may increase susceptibility to the effects of traffic air pollution on other health-related outcomes, such as cardiovascular or respiratory disease (Clougherty and Kubzansky 2009), no information is currently available to identify those individuals who may suffer worse cognitive damage from air pollution exposure.

Telomeres are regions of repetitive DNA at the ends of chromosomes that protect from DNA rearrangements and chromosomal end-to-end fusions and have established roles in biological aging (Blackburn 2001). Telomere length (TL) has been shown to decrease non-linearly with age across different tissues in living organisms, including blood leukocytes (Saretzki and Von Zglinicki 2002, Young 2010). Shorter leukocyte TL has been associated with age-related

diseases, such as cardiovascular diseases (Fyhrquist et al. 2011), cancer (Artandi and DePinho 2010) and cognitive impairment (Roberts et al. 2014), as well as for mortality (Svenson et al. 2009), although one study reported opposite associations (Sanchez-Espiridion et al. 2014).

However, experimental data have shown that blood leukocytes carrying longer telomeres are capable of building stronger inflammatory responses (Weng et al 1998). Cells with longer telomeres, indeed, have higher capacity for rapid proliferation and clonal expansion (Hodes et al. 2002), which is key for inflammatory cells to generate inflammatory responses. Inflammation is believed to play a central role in the effects of traffic air pollution, including those on cognitive function (Schram et al. 2007). Therefore, individuals with longer blood TL may be more susceptibile to the adverse cognitive effects of air pollution. To the best of our knowledge, however, whether individuals with longer blood TL have differential susceptibility to BC exposure has never been investigated in relation to cognitive function.

In this study of older individuals participating in the United States (U.S.) Veteran Affairs (VA) Normative Aging Study (NAS), we measured blood TL, BC exposure, cognitive function, and C-reactive protein (CRP) levels. We examined whether the previously observed negative association of BC exposure with cognitive function (Power et al. 2011) was stronger in men with longer blood cell TL. To further clarify the role of inflammation, we also determined whether the association between BC exposure and cognition was stronger in men with higher levels of C-Reactive Protein (CRP), a marker of systemic inflammation.

METHODS

Study sample

The NAS is an ongoing longitudinal study of aging in men from eastern Massachusetts established in 1963 by the U.S. Department of VA (Bell et al. 1966). In total, 1596 individuals free of chronic disease at recruitment were invited to undergo an in-person examination every 3–5 years since 1984. Participants provided information on medical history, lifestyle, and demographic factors and underwent a physical examination and laboratory tests, at each visit. Starting in 1993, all participants underwent cognitive tests (Weisskopf et al. 2004). Collection of blood samples for molecular analysis, such as TL, began in January 1999 (Baccarelli et al. 2010; McCracken et al. 2010). We included in the present analyses all cognitive assessments (up to 3; average=1.3 visits per participant) performed at the time of first blood collection or later. Each study visit for which we had TL, CRP, cognitive assessment, and information on confounding covariates was included in the study. Participants provided written informed consent at each visit, and the VA Boston Healthcare System Institutional Review Board approved the study. Participants who had experienced a stroke before the first cognitive assessment on or after January 1, 1999 (3.21% of individuals) were excluded from the study. Out of the initial 814 active participants in the NAS between 1999-2007, i.e. during the period for which TL measurements were performed, a total of 428 men with complete TL measurements, exposure assessments, cognitive testing and covariate data were included in the analysis (Table S1). Compared to participants included in the analysis, those excluded were older (mean age±SD of 73.6±6.6 vs. 74.3±6.8 years, respectively, p=0.05), but BC, TL and CRP levels were not significantly different (p=0.18, 0.21 and 0.08, respectively).

Cognitive testing

At each visit, participants completed the Mini-Mental State Examination (MMSE), a validated global cognitive test to screen for dementia (Tombaugh and McIntyre 1992). MMSE assesses several cognitive domains, such as orientation, immediate and short-term recall, attention and calculation, word finding, construction reading and writing skills, and ability to follow a three-step command. The range of scores is 0 to 30; however, the maximum score in this study was 29 due to exclusion of a county identification question because the general population in Massachusetts is not knowledgeable of counties (Weisskopf et al. 2004). Cognitive data were considered from study visits between 1999 and 2007.

Exposure assessment

BC exposure at each participant's address was estimated using a validated spatio-temporal regression model for the greater Boston, Massachusetts, area that predicts daily BC levels starting on January 1, 1995. The model is based on data from 148 monitoring sites, daily BC concentrations at a central monitor, and predictors based on meteorological conditions (e.g., wind speed), measures of land use (e.g., traffic-density), and other descriptors (e.g., day of the week) at each monitor location. The validation steps included checks on robustness, graphical convergence and goodness of fit of the model (Gryparis et al. 2007). BC estimates obtained from this model were considered a surrogate for individual exposure to traffic-related air pollution at

residential addresses. Average exposure during the year before each visit was estimated by taking the average of the 365 daily BC estimates at the participant's residential address before the date of each cognitive assessment, as reported previously (Power et al. 2011). These estimates were used as proxy measures of long-term BC exposure due to the high correlation with averages of BC over longer time windows (Table S2).

Blood measurements: Telomere length and C-reactive protein

Seven mL of whole blood were collected by venous phlebotomy in EDTA tubes. Buffy coat was obtained from the blood samples and stored at -20°C until DNA isolation using a QiAmp DNA blood kit (Qiagen, Germantown, MD, USA). Leukocyte TL was measured by quantitative real-time polymerase chain reaction (qRT-PCR), as described below. Relative leukocyte TL was measured by determining the ratio of the telomere repeat (T) copy number to a single copy gene (S) copy number (T:S ratio) in a given sample. Human beta-globin was used as the single copy gene. To control for plate effects, leukocyte TL was calculated as relative units, expressing the ratio between leukocyte TL in the test DNA and leukocyte TL in a DNA pool used to generate a standard curve in each PCR. The standard pool comprised DNA from participants randomly selected from NAS (50 ng per sample) and was used in each run to create a standard curve, which ranged from 20 to 0.25 ng/μL of pooled DNA. An eight-point standard curve ranging from 30 to 0.234 ng/μL and derived from the serially diluted pooled DNA was included in each PCR plate so that relative quantities of T and S could be determined.

For each sample, we prepared a 25-μL mixture of DNA sample (2 ng/μL) and carrier *Escherichia coli* DNA (15 ng/μL) to increase PCR reproducibility. These mixtures were heated

to 96°C × 10 min and then cooled to room temperature. PCR primer sets for T and S and the PCR mix composition were previously described (Hou et al. 2009). Using a MICROLAB STARlet Robot (Hamilton Life Science Robotics, Bonaduz, Switzerland), we transferred 4 ng of DNA in a 5-μL reaction mix into 384-well plates. We performed PCR runs on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). After amplification, product specificity was confirmed by dissociation curve analysis. We ran all samples in triplicate, and the average of three T measurements was divided by the average of three S measurements to calculate average T:S ratio (Farzaneh-Far et al. 2008). To test reproducibility of this method, we amplified T and S in 15 samples in triplicate on three consecutive days. The coefficient of variation for the average T:S ratio of samples analyzed over three consecutive days was 8.7%, similar to the reproducibility originally reported for this method (Cawthon 2002).

We analyzed high-sensitivity CRP on fasting blood samples in duplicate and in a single batch to avoid between-batch analytical variation. The performance of the assays was monitored with standard quality control procedures including the analysis of quality control samples in each batch. CRP was measured in serum using immunoturbidimetric assays on a Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN, USA) with reagents and calibrators from Denka Seiken (Niigata, Japan) (McCracken et al. 2010). The high-sensitivity CRP analysis was done in Rifai's laboratory at Boston Children's Hospital, and the run-to-run CVs, at different hs-CRP-concentrations (0.47-54.9 mg/L) varied between 6.4%-2.9% (Rifai et al. 1999).

Statistical analysis

We used SAS (V9.2; SAS Institute Inc., Cary, NC, USA) for all analyses. The dependent variable was MMSE score of each participant in each visit. This variable presented a ceiling effect due to 15.43% of observations reaching the maximum score of 29 and 9.65% showing scores smaller than or equal to the typical screening cut-off score for dementia (MMSE = 24) (Weisskopf et al. 2004). To take into account this distribution, MMSE scores were dichotomized in all analyses as >25 and ≤25. The ≤25 MMSE score category was considered low cognitive performance. We described the association among TL, chronological age, CRP, and BC concentration using Pearson's correlations.

TL measurements, considered at each visit as a proxy of biological aging, were categorized into quintiles. This approach accommodates potential non-linear associations. We estimated the main effects of both BC levels and TL on the odds of low MMSE score (≤25) using logistic regression models with generalized estimating equations using independent correlation structure and empirical variance estimates to avoid bias and account for repeated BC levels and MMSE scores. Because the relationship between BC levels and the odds of low MMSE scores was log-linear (Power et al. 2011), we used log-transformed BC (ln(BC)) in all analyses:

$$\ln(p_{ij}/(1-p_{ij}))=\beta_0+\beta_1*(\ln(BC_{ij}))+\beta_2*X_{2ij}+\ldots+\beta_n*X_{nij} \quad [1]$$

where p is the proportion with low MMSE scores; β_0 is the overall intercept; β_1 is the regression coefficient representing the predicted ln-odds of a low MMSE score with a 1-unit increase in

natural log-transformed BC concentration; $\beta_2 \ldots \beta_n$ are the regression coefficients for n covariates included in adjusted models; i=1, 2,…,428 represents the subject; and j=1, 2, 3 represents the j^{th} cognitive assessment.

To evaluate whether TL modified the association of BC exposure with cognitive function, we added to model [1] interaction terms for BC exposure and indicator terms for TL quintiles. We considered the lower-order TL term as reference level. To determine whether the effect modification by TL quintiles was independent of the interaction of either age or CRP levels with BC levels, we fitted additional models that included two sets of interaction terms—either BC exposure and age, or BC exposure and CRP levels—to the model with the interaction term for BC exposure and TL. Both age and CRP level were categorized by quintiles to facilitate interpretation and to evaluate non-linear association.

All models were adjusted for potential confounders or predictors of cognitive function, including age at cognitive assessment (continuous), CRP level (continuous), and the following variables measured at baseline, such as education (<12, 12–16, >16 years), first language (English/not English), computer experience (yes/no), physical activity [<12, 12–30, ≥30 metabolic equivalent hours/week], body mass index (<25, ≥25 kg/m^2), dark-meat fish consumption (<once/week , ≥once/week), alcohol intake (<2, ≥2 drinks/day), smoking status (never, current, former), percentage of the participant's census tract that is nonwhite, percentage of the adult residents in the participant's census tract with at least a college degree, whether cognitive data were from the

participant's first cognitive assessment (yes/no), whether the participant was a part-time resident of the greater Boston area (yes/no), hypertension (yes/no), diabetes (yes/no), and coronary heart disease (CHD) (yes/no). Census variables were collected in 2000 and health conditions were diagnosed by physicians during each visit. Covariates selection was based on previous literature (Power et al. 2011) and association with the outcome. Results were considered noteworthy at $p<0.05$.

We conducted a set of sensitivity analyses to confirm robustness of our findings. We performed analyses excluding MMSE outliers, identified as continuous cognitive scores deviating three interquantile ranges from the first or third quartile, and observations with CRP levels indicative of an active immune response (CRP levels ≥ 10mg/L) (Ridker 2003). In additional sensitivity analyses, we excluded hypertension, diabetes and CHD from the list of covariates, as these variables may act as mediators of the BC-cognition relationship.

RESULTS

Characteristics of participants and BC exposure levels

Demographic and clinical characteristics of the cohort of 428 men at first cognitive assessment are shown in Table S3. Participants were 56–94 years of age with a mean±SD of 73.6±6.6 years. Most individuals had at least some college or graduate-level education (71.03%), reported alcohol consumption of less than two drinks per day (78.27%), and were not affected by diabetes

mellitus (82.24%) or coronary heart disease (70.79%)(Table S3). Mean TL was 1.26 relative units (SD=0.51) at the first visit, and TL was negatively correlated with age (Pearson's r=-0.13, *p*=0.01), showing a decrease over subsequent visits (Table S4). Out of 428 individuals in this analysis, 21 had three cognitive assessments, 152 had two and 255 had only one cognitive exam. The proportion of men with a low MMSE score increased from 18.7% at the first visit to 22.0% and 28.6% for men who completed a second and third visit, respectively (Table S4).

On the natural scale, 1-year average BC exposure estimates ranged between 0.02–1.90 $\mu g/m^3$ (mean±SD=0.46±0.23 $\mu g/m^3$) and exhibited a right-skewed distribution. Due to the skewed distribution, we log-transformed BC and reported associations for a doubling in BC concentration on the natural scale, or approximately a 0.69-unit change in ln(BC). BC exposure showed no significant correlation with age (Pearson's r=-0.04, *p*=0.41) or TL (Pearson's r=-0.02, *p*=0.62). Distribution of MMSE scores, BC levels, TL and CRP measurements according to classes of age are shown in Table S4.

BC exposure, TL, CRP levels, and cognitive function

BC exposure was significantly associated with higher relative odds of low MMSE scores (Table 1). A doubling of the average BC concentration during the previous year was associated with 1.57 times (95% CI: 1.20, 2.05) higher relative odds of low MMSE scores based on the covariate-adjusted model, consistent, but not identical, due to moderately different sample size,

with previous estimates for this cohort (multivariable adjusted OR=1.3, 95% CI: 1.1, 1.6 for a doubling of BC level during the previous year) (Power et al. 2011). TL, both continuous and categorized in quintiles, showed no significant association with low MMSE scores (Table 1). However, TL significantly modified the association between BC exposure and MMSE score. In particular, BC exposure had a significantly stronger association with MMSE scores in men with longer telomeres (5^{th} quintile) (OR=3.23; 95% CI: 1.37, 7.59; p=0.04 for BC-TL interaction; Wald test: p=0.03), while this association was null in men belonging to the other quintiles (Table 2).

Because TL negatively correlates with chronological age, we also fitted models that included interaction terms between BC exposure and quintiles of age in addition to the interaction terms between BC exposure and TL quintiles. The MMSE-BC relationship was evaluated using two reference groups: the 5^{th} age quintile and the 1^{st} TL quintile. This model showed no significant interaction (p>0.10) between age and BC exposure for any age group (Table 3). However, this model confirmed the interaction between TL and BC exposure and showed a substantially stronger association of BC exposure with relative odds of lower MMSE scores only among individuals with longer TL (5^{th} quintile) and older age (5^{th} quintile) (OR=2.49; 95% CI: 1.07, 5.76; p=0.04 for BC-by-TL interaction; Wald test: p=0.13) than in those with shorter TL and older age (Table 3). In addition a doubling of BC levels was associated with 2.49 times (95% CI: 1.07, 5.76) higher relative odds of low MMSE scores among men within the 5^{th} TL quintile and 5^{th} age quintile. Only 17 participants (4%) were part-time residents of the greater Boston and their exclusion did not change the results significantly (data not shown).

To elucidate the role of inflammation in modifying the association between BC and cognitive function, we evaluated the influence of CRP levels on the association between BC exposure and MMSE scores. CRP levels were not correlated with TL (Pearson's r=-0.01, p=0.79), but they significantly modified the association of BC exposure with MMSE scores (Table 4). In this model the MMSE-BC relationship was evaluated using the 1st CRP quintile and the 1st TL quintile as reference levels. BC exposure had a stronger association with MMSE scores among participants with higher CRP levels (5th quintile) (OR=2.68; 95% CI: 1.06, 6.79; p=0.04 for BC-by-CRP interaction) (Table 4). BC exposure showed a substantially stronger association with relative odds of lower MMSE scores among individuals with longer TL (5th quintile) and lower CRP levels (1st quintile) (OR=2.18; 95% CI: 0.77, 6.23; p=0.03 for BC-by-TL interaction; Wald test p=0.05) than those with shorter TL and lower CRP levels (Table 4).CRP levels were not associated with MMSE scores (Table S5). The BC-MMSE association did not show a monotonic pattern with quintiles of TL, age and CRP.

The BC-MMSE associations were similar to the main analyses when we excluded men with higher CRP levels and MMSE influential values and outliers (10 men) (Tables S6–S8) and when we did not adjust for diabetes, hypertension, and CHD (Tables S9-S11).

DISCUSSION

In a cohort of older urban residents, we observed that the association between 1-year exposure to traffic-related air pollution—as traced by time- and space-resolved estimates of BC levels—and global cognition may be augmented in individuals with longer leukocyte TL even adjusting for chronological age and CRP levels. We also showed that participants with higher CRP levels and lower TL quintile may exhibit stronger BC-MMSE associations. In addition the BC-MMSE relationship did not show a monotonic pattern according to both TL and CRP quintiles. In the NAS cohort, BC level was previously associated with higher relative odds of impaired cognition (Power et al. 2011). Because we excluded participants without TL measurements, estimates presented here are moderately different from those previously published (Power et al. 2011). Epidemiological studies have evaluated the association between air pollution and cognition in different populations, including children (Freire et al. 2010), adults (Chen and Schwartz 2009) and older individuals (Power et al. 2011; Ranft et al. 2009; Weuve et al. 2012). Although those studies collectively suggest a negative impact of air pollution on cognitive function, whether biological factors, such as TL, age, and inflammation, modify this association had not been studied.

In human studies particulate pollution has been showed to induce oxidative damage and inflammation (Araujo 2011) and BC-rich particles has been showed to cause both lung and systemic inflammation (Highwood and Kinnersley 2006). Inflammation has been extensively associated with dementia and cognitive decline in the elderly (Gorelick 2010). Also, inflammatory molecules, such as cytokines, chemokines, and complement factors, have been

found in cerebrospinal fluid and beta-amyloid plaques in patients with Alzheimer's disease (AD). Some evidence suggests that beta-amyloid and neurofibrillary tangles could provide the inflammatory stimuli to microglia in AD (Gorelick 2010).

Leukocytes have a key role in inflammation as they coordinate all responses to antigen challenges in the human body (Granger and Senchenkova 2010). Our finding of a stronger association between BC and lower cognition in individuals with longer TL is consistent with the hypothesis that leukocytes with longer TL mount stronger inflammatory systemic responses (Dioni et al. 2011; Hou et al. 2012). Increased systemic inflammatory responses to traffic-derived pollution may induce stronger effects on systemic targets (Fang et al. 2012), including the brain (Arfanakis et al. 2013). This hypothesis is particularly plausible because the stronger association between BC and cognition was found only in participants with TL at the highest quintile, while no dose-response relationship was found at lower TL. This pattern of associations—which we also observed for the interaction between BC and CRP in this study—suggests the existence of an activation threshold, which is typical in inflammatory responses, particularly if they—such as those elicited by traffic air pollution— are induced through the NF-kB pathway (Nam et al. 2009). Of note, a previous study described an L-shaped association of TL with mild cognitive impairment; in particular, TL in the top quintile, but not in other TL quintiles, was associated with relative odds of both prevalent and incident mild cognitive impairment (Roberts et al. 2014). Alternatively, our finding might reflect a saturation effect among individuals with shorter TL. Those individuals may have experienced high levels of oxidative damage and/or have higher background levels of oxidative stress. Due to this high

oxidative state, BC exposure might only produce incremental oxidative damage in these individuals.

Inflammatory markers, including circulating CRP, have been associated with risk of dementia and cognitive decline in the elderly (Marioni et al. 2009). Neither TL nor MMSE score were significantly associated with CRP levels here. However, we found that only higher CRP levels modified the association between BC exposure and cognitive function, supporting that traffic-related inflammatory response may induce stronger effects on systemic targets. To our knowledge, this is the first observation of the role of CRP as a modifier of the association between BC exposure and cognitive function in the elderly.

One limitation of this study is that it is based on relative TL rather than absolute TL. However, previous comparisons show that qRT-PCR-measured relative TL correlates well with absolute TL (Ehrlenbach et al. 2009). Another limitation is that these findings are based on a cohort of older white men and may apply only to populations with similar characteristics. Also, NAS has a limited chronological age range (56–94 years), with 50% of participants aged between 69–78 years. This limited age range may have contributed to the lack of association between TL and cognitive measures (Devore et al. 2011; Martin-Ruiz et al. 2006; Valdes et al. 2010; Yaffe et al. 2011). Finally, BC exposure was determined using geospatial models to estimate traffic-related pollution, so these estimates may differ from actual levels of personal exposure. However, as described previously, this approach is expected to produce non-differential misclassification and

is highly unlikely to bias results away from the null, but rather to underestimate the observed associations (Kioumourtzoglou et al. 2014). Further, BC concentrations are highly spatially heterogeneous due to numerous local sources, so the models used here are expected to provide sufficient contrast.

CONCLUSIONS

Our findings indicate that TL modifies the relationship of BC exposure with cognitive impairment observed in older men. High CRP levels were also predictive of higher susceptibility. Further research is warranted to confirm the findings of the present study in other study populations as well as to evaluate whether TL modifies the effects of BC exposure on other target systems, such as cardiovascular or respiratory effects. These results increase our understanding of the role of biological factors in the response to environmental stressors in age-related cognitive impairments.

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Table 1. Relative odds of low Mini-Mental State Examination (MMSE) score (≤25)[a] associated with black carbon (BC) levels[b] and with telomere length (TL), continuous and in quintiles.

Association with MMSE	OR	95% CI	*P*	Cases	Non-cases
BC concentration[a,b]	1.57	(1.20, 2.05)	0.001	124	498
TL (as continuous variable)[a]	0.97	(0.62, 1.52)	0.88	124	498
TL in quintiles[a]					
TL 1st quintile (0.30–0.79)	Ref.	.	.	28	96
TL 2nd quintile (0.80–1.04)	0.94	(0.52, 1.71)	0.84	25	99
TL 3rd quintile (1.05–1.25)	0.90	(0.46, 1.78)	0.77	22	103
TL 4th quintile (1.26–1.56)	1.33	(0.67, 2.66)	0.42	27	97
TL 5th quintile (1.57–3.81)	0.89	(0.43, 1.82)	0.75	22	103
Test for linear trend			0.79	124	498

OR=odds ratio, 95% CI=95% confidence interval

[a] Adjusted for age (continuous), education level, first language, computer experience, physical activity level, body mass index, dark-meat fish consumption, alcohol consumption, smoking status, percent of adults with a college degree, percentage of the participant's census tract that is nonwhite, indicator for first cognitive assessment, indicator for part-time resident, hypertension, diabetes, coronary heart disease, and C-reactive protein levels (continuous).

[b] Association with each doubling in BC level, corresponding to a 0.69 $\mu g/m^3$ increase in average ln(BC) concentration

Table 2. Modification by quintiles of telomere length (TL) of the relative odds of low Mini-Mental State Examination (MMSE) score (≤25)[a] associated with black carbon (BC) levels[b]

Association between BC and low MMSE (≤25) by TL	OR	95% CI	*p*	*p* for interaction[c]	Cases	Non-cases
TL 1st quintile (0.30–0.79)	1.26	(0.83, 1.90)	0.27	.	28	96
TL 2nd quintile (0.80–1.04)	1.45	(0.96, 2.19)	0.08	0.62	25	99
TL 3rd quintile (1.05–1.25)	1.80	(0.89, 3.64)	0.10	0.39	22	103
TL 4th quintile (1.26–1.56)	1.05	(0.63, 1.75)	0.85	0.57	27	97
TL 5th quintile (1.57–3.81)	3.23	(1.37, 7.59)	0.01	0.04	22	103
All interaction terms at once (Wald test)				0.03	124	498

OR=odds ratio; 95% CI=95% confidence interval

[a] Adjusted for age (continuous), education level, first language, computer experience, physical activity level, body mass index, dark-meat fish consumption, alcohol consumption, smoking status, percent of adults with a college degree, percentage of the participant's census tract that is nonwhite, indicator for first cognitive assessment, indicator for part-time resident, hypertension, diabetes, coronary heart disease, and C-reactive protein levels (continuous).

[b] Association with each doubling in BC level, corresponding to a 0.69 $\mu g/m^3$ increase in average ln(BC) concentration

[c] TL-by-BC level interaction

Table 3. Modification by quintiles of telomere length (TL) and age of the relative odds of low Mini-Mental State Examination (MMSE) score (≤25)[a] associated with black carbon (BC) levels.[b]

Association between BC and low MMSE (≤25) by TL or Age	OR	95% CI	*p*	*p* for interaction	Cases	Non-cases
TL 1st quintile (0.30–0.79)	1.07 [e]	(0.56, 2.08)	0.84	.	28	96
TL 2nd quintile (0.80–1.04)	1.29 [e]	(0.71, 2.35)	0.42	0.54 [c]	25	99
TL 3rd quintile (1.05–1.25)	1.58 [e]	(0.73, 3.45)	0.25	0.35 [c]	22	103
TL 4th quintile (1.26–1.56)	0.92 [e]	(0.42, 2.02)	0.84	0.67 [c]	27	97
TL 5th quintile (1.57–3.81)	2.49 [e]	(1.07, 5.76)	0.03	0.04 [c]	22	103
Age 1st quintile (56–67)	1.46 [f]	(0.51, 4.17)	0.49	0.54 [d]	13	102
Age 2nd quintile (68–70)	1.59 [f]	(0.51, 4.96)	0.44	0.43 [d]	14	73
Age 3rd quintile (71–74)	0.93 [f]	(0.40, 2.15)	0.87	0.62 [d]	24	139
Age 4th quintile (75–78)	1.43 [f]	(0.60, 3.41)	0.43	0.48 [d]	19	90
Age 5th quintile (79–94)	1.07 [f]	(0.56, 2.08)	0.84	.	54	94
All interaction terms at once (Wald test)				0.13	124	498

OR=odds ratio; 95% CI=95% confidence interval

[a] Adjusted for education level, first language, computer experience, physical activity level, body mass index, dark-meat fish consumption, alcohol consumption, smoking status, percent of adults with a college degree, percentage of the participant's census tract that is nonwhite, indicator for first cognitive assessment, indicator for part-time resident, hypertension, diabetes, coronary heart disease, and C-reactive protein levels (continuous).

[b] Association with each doubling in BC level, corresponding to a 0.69 $\mu g/m^3$ increase in average ln(BC) concentration

[c] *p*-values for TL-by-BC level interaction

[d] *p*-values for age-by-BC level interaction

[e] ORs for a doubling BC increase according to TL quintiles with Age 5th quintile as reference level

[f] ORs for a doubling BC increase according to Age quintiles with TL 1st quintile as reference level

Table 4. Modification by quintiles of telomere length (TL) and C-reactive protein (CRP) of the relative odds of low Mini-Mental State Examination (MMSE) score (≤25)[a] associated with black-carbon (BC) levels[b].

Association between BC and low MMSE (≤25) by TL or CRP	OR	95% CI	*p*	*p* for interaction	Cases	Non-cases
TL 1st quintile (0.30–0.79)	0.77[e]	(0.35, 1.71)	0.53	.	28	96
TL 2nd quintile (0.80–1.04)	1.07[e]	(0.44, 2.63)	0.89	0.34[c]	25	99
TL 3rd quintile (1.05–1.25)	1.28[e]	(0.48, 3.45)	0.64	0.32[c]	22	103
TL 4th quintile (1.26–1.56)	0.78[e]	(0.29, 2.09)	0.64	0.97[c]	27	97
TL 5th quintile (1.57–3.81)	2.18[e]	(0.77, 6.23)	0.14	0.03[c]	22	103
CRP 1st quintile (0.04–0.66)	0.77[f]	(0.35, 1.71)	0.53	.	24	99
CRP 2nd quintile (0.67–1.20)	1.54[f]	(0.72, 3.31)	0.27	0.16[d]	23	102
CRP 3rd quintile (1.21–2.04)	1.02[f]	(0.35, 2.96)	0.97	0.45[d]	32	92
CRP 4th quintile (2.04–3.99)	0.70[f]	(0.24, 2.04)	0.52	0.84[d]	24	100
CRP 5th quintile (4.00–72.20)	2.68[f]	(1.06, 6.79)	0.04	0.04[d]	21	105
All interaction terms at once (Wald test)				0.05	124	498

OR=odds ratio; 95% CI=95% confidence interval

[a] Adjusted for age (continuous), education level, first language, computer experience, physical activity level, body mass index, dark-meat fish consumption, alcohol consumption, smoking status, percent of adults with a college degree, percentage of the participant's census tract that is nonwhite, indicator for first cognitive assessment, indicator for part-time resident, hypertension, diabetes, and coronary heart disease.

[b] Association for each doubling in BC level, corresponding to a 0.69 $\mu g/m^3$ increase in average ln(BC) concentration

[c] *p*-value for TL by BC level interaction

[d] *p*-value for CRP level by BC level interaction

[e] ORs for a doubling BC increase according to TL quintiles with CRP 1st quintile as reference level

[f] ORs for a doubling BC increase according to CRP quintiles with TL 1st quintile as reference level